Page 12: remove the fourth paragraph of page 11 through to the first line of page 12, and insert therefor:

Additionally, the plasmids may contain one or more transaction control sequences. One such sequence should be found within the transposable sequence, such that when the transposon hops into the target plasmid, it carries along with it the transcription control sequence. An exemplary sequence is the T7 promoter, but any promoter or enhancer that is functional in prokaryotic cells may be used. Useful promoters include, but are not limited to, *lac* (*E. coli*), *trp* (*E. coli*), *araBAD* (*E. coli*), *tac*, hybrid, (*E. coli*), *trc*, hybrid (*E. coli*), *lpp-lac* hybrid (*E. coli*), PL (λ), *T7-lac* operator and λPL, PT7 (λ, T7).

Page 19: remove the first paragraph and insert therefor:

Example 2: Construction of BAC vectors

1. BACTAPUC1 (pBTP1)- The first version of the vector, pBTP1, combines pBeloBAC with a high copy PUC-based vector. As shown in Figure 7, insertion of an entire PUC plasmid into the cloning site accomplishes several things. First, it simplifies the purification of the vector prior to cloning by virtue of the high copy ori within the PUC insert which drives the copy number to >100 copies/cell. Second, by using a unique oligonucleotide adapter, we have introduced additional cloning sites. This includes the ability to utilize cloning based on single base extensions. Thermostable polymerases such as Taq have a nontemplate-dependent activity which adds a single deoxyadenosine (A) to the 3' end of DNA. This single extended DNA will ligate efficiently with a vector that has corresponding deoxythymidine (T) ends. By incorporating a restriction site with internal degenerate internal bases, such as AhdI

(GACNNNNNGTC (SEQ ID NO:1)), we can create a vector which, when cut with AhdI, leaves a single T on each end. By treating the genomic DNA with a series of polymerases (T4 and Klenow for blunting followed by Taq to add a single A) DNA can be directly cloned without the need for partial restriction digestion. This latter point is key since cloning by partial restriction digestion will decrease the average insert size of the library by at least half (see below).

A2

Page 20: Remove first paragraph and insert therefor:

to cloning will decrease the average insert size of the final library. In addition, since the average size of the input DNA is in the range of 150 kb before digestion and drops to 75 kb after partial digestion, it is likely that an increasing bias will occur as we attempt to clone fragments above 80-100kb. This will be dependent on the enzyme used for digestion and the number of sites in the DNA. Therefore, alternate strategies for cloning directly become key in constructing high quality libraries (see Table 1). The single base extension cloning system described above is one way to circumvent this problem. However, although the efficiency of cloning is greater than blunt-end cloning, it is not as high as with multiple base ligation. Also, the addition of the A tail is not 100% efficient, so not all DNA will be ligatable. An alternate approach is to incorporate non-palidromic adapters with 4-base pair overhangs which will greatly increase the efficiency of cloning. Figure 9 (pBTP3) illustrates an example of one such system which uses a second degenerate restriction enzyme, BstXI (CCANNNNNNTGG) (SEQ ID NO:2). In this system, adapters with non-homologous ends (5' CACA 3') are ligated onto blunt-end genomic DNA. These adapters will not self-ligate but will only anneal with corresponding ends which are generated in the vector by inserting the appropriate BstXI restriction site (5' GTGT 3').

A3